# Intestinal infection at onset of mycophenolic acid-associated chronic diarrhea in kidney transplant recipients

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Abstract: BACKGROUND: Chronic diarrhea after kidney transplantation is often attributed to mycophenolic acid (MPA) toxicity. We hypothesize that intestinal infections contribute to the pathogenesis of chronic MPA-associated diarrhea. METHODS: In this retrospective study, all patients (n = 726) receiving a kidney transplant between 2000 and 2010 at the University Hospital Zurich were followed until July 2014 for occurrence of chronic diarrhea ( 4 weeks). Infectious triggers at diarrhea onset were assessed by reviewing medical history, stool microbiology, and histology of colon biopsies. RESULTS: In 46 patients (6.3% of the cohort), a total of 51 episodes of chronic diarrhea during MPA treatment were documented. The diarrhea episodes were often severe, as confirmed by significant weight loss. The cumulative incidence of chronic diarrhea was uniformly distributed throughout the post-transplant period, with 2.0%, 5.1%, and 9.6% at 1, 5, and 10 years, respectively. Evidence was found for intestinal infection at diarrhea onset in 38 episodes (74.5%). Occurrence of diarrhea onset showed a seasonal distribution with peaks in April and October/November. Specific antimicrobial treatment alone was associated with a 19% resolution rate only, whereas combination with dose reduction of MPA or switch from mycophenolate mofetil to enteric-coated mycophenolate sodium resulted in a 22.7% and 76.5% resolution rate, respectively. Change to an MPA-free regimen was associated with a 100% resolution rate. CONCLUSION: These results provide first evidence for a contribution of intestinal infections in chronic post-transplant diarrhea associated with MPA treatment.

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**Abstract:** *Background.* Chronic diarrhea after kidney transplantation is often attributed to mycophenolic acid (MPA) toxicity. We hypothesize that intestinal infections contribute to the pathogenesis of chronic MPA-associated diarrhea.
*Methods.* In this retrospective study, all patients ($n$ = 726) receiving a kidney transplant between 2000 and 2010 at the University Hospital Zurich were followed until July 2014 for occurrence of chronic diarrhea (≥4 weeks). Infectious triggers at diarrhea onset were assessed by reviewing medical history, stool microbiology, and histology of colon biopsies.
*Results.* In 46 patients (6.3% of the cohort), a total of 51 episodes of chronic diarrhea during MPA treatment were documented. The diarrhea episodes were often severe, as confirmed by significant weight loss. The cumulative incidence of chronic diarrhea was uniformly distributed throughout the post-transplant period, with 2.0%, 5.1%, and 9.6% at 1, 5, and 10 years, respectively. Evidence was found for intestinal infection at diarrhea onset in 38 episodes (74.5%). Occurrence of diarrhea onset showed a seasonal distribution with peaks in April and October/November. Specific antimicrobial treatment alone was associated with a 19% resolution rate only, whereas combination with dose reduction of MPA or switch from mycophenolate mofetil to enteric-coated mycophenolate sodium resulted in a 22.7% and 76.5% resolution rate, respectively. Change to an MPA-free regimen was associated with a 100% resolution rate.
*Conclusion.* These results provide first evidence for a contribution of intestinal infections in chronic post-transplant diarrhea associated with MPA treatment.

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Key words: mycophenolic acid-related diarrhea; chronic post-transplant diarrhea; infectious trigger; intestinal infection

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Chronic diarrhea after kidney transplantation is common, yet often neglected despite substantially affecting quality of life (1) and graft and patient survival (2–5). The cause of chronic diarrhea in transplant recipients has typically been attributed to drug toxicity (5), especially related to mycophenolic acid (MPA) (6). However, growing evidence indicates that infections are involved as causative factors of chronic diarrhea in immunosuppressed patients, with a particular role for gastrointestinal (GI) viral infections (7–11). The balance, resulting from the interaction between pathogens, intestinal flora, immune system, and pharmacological therapy, merits further investigation.

MPA was introduced into clinical transplantation in 1995 (12) and has become a first-line immunosuppressive drug. It is part of the standard therapy in solid organ transplantation and is overall well tolerated. Two therapeutically equivalent preparations (13, 14) are currently on the market: (i) mycophenolate mofetil

(MMF, Cellcept®), a prodrug that needs to be converted into MPA, and (ii) the enteric-coated mycophenolate sodium (EC-MPS, Myfortic®) (15), developed to reduce GI side effects (16). In both cases, MPA is the active drug, which acts as a non-competitive inhibitor of inosine-5′ monophosphate dehydrogenase, the rate-limiting enzyme in *de novo* purine synthesis. As a result, MPA suppresses DNA synthesis and cell proliferation with a relative selectivity for B and T cells, as lymphocytes are completely inosine-5′ monophosphate-dehydrogenase-dependent for *de novo* purine synthesis. In contrast, enterocytes, even though rapidly dividing, are less susceptible to the effects of MPA, as the salvage pathway for purine synthesis is active in these cells. Nevertheless, colon biopsies from patients treated with MPA and suffering from chronic diarrhea show prominent anti-proliferative effects on the GI mucosa (6, 17). Histologically, 2 different morphologic patterns can be distinguished: (i) predominant crypt distortion, also called inflammatory bowel disease-like MPA-associated toxicity, and (ii) predominant apoptosis, also called graft-versus-host-like MPA-associated toxicity (6). Because chronic diarrhea generally is recognized as a common side effect of treatment with MPA, clinical practice consists of dose reduction or switch of immunosuppression. Dose reduction, however, is not without risk, as a reduced dose of MMF has been associated with increased risk for acute graft rejection (relative risk increase by 4% for every week that the MMF dose was reduced below the full dose) (18). Whether switch of immunosuppression from MMF to EC-MPS helps reducing diarrhea symptoms is a matter of debate. Diverse open-labeled studies reported reduced lower GI side effects with EC-MPS compared with MMF (16, 19), but this benefit was not confirmed in randomized, blinded trials showing an incidence of diarrhea at 1 year of 5% for both drug formulations (13, 14). In most centers, a switch from MPA to azathioprine (AZA) (Imurek®) is usually avoided because of reported reduced graft survival with AZA as compared to MMF (12), although this approach is safe in the short-term (20).

The exact mechanism of MPA-associated colitis remains unknown. Direct cytotoxicity leading to inflammation and suppressed intestinal regeneration, as well as dysregulation of the balance between immunity and tolerance against luminal antigens, have been proposed (6, 15, 17). Here, we show that an infectious agent is detected in the majority of patients at onset of diarrhea, suggesting that intestinal infections might be involved in the pathophysiology of MPA-associated chronic diarrhea.

## Methods

### Study design and population

We performed a single-center retrospective, observational cohort study. We screened all adult renal allograft recipients transplanted at the University Hospital Zurich (Switzerland) between January 2000 and December 2010 for occurrence of chronic diarrhea until July 2014. All patients were seen at least annually in the transplant center and at regular intervals by the local nephrologists. In case of graft dysfunction or other clinically relevant problems, patients were referred to the transplant center more frequently. Immunosuppression therapy was administered according to our internal guidelines. Induction therapy with basiliximab or anti-thymocyte globulin was included in patients at high risk for rejection. Maintenance immunosuppression consisted mostly of a calcineurin inhibitor (cyclosporine or tacrolimus) and an anti-proliferative drug (usually MPA). In most cases, steroids were withdrawn 6 months after transplantation. Cytomegalovirus (CMV) prophylaxis or preemptive monitoring was performed according to the individual patient risk profile (21).

To screen for chronic diarrhea, the standardized electronic patient files were reviewed for episodes of diarrhea since transplantation. Adult patients (>18 years of age) during immunosuppression with MPA at diarrhea onset and suffering from chronic diarrhea, defined as diarrhea lasting for at least 1 month according to the Centers for Disease Control and Prevention classification (22), were included. Patients with combined solid organ transplantation were excluded from the study. Patients with pre-existing GI diseases (e.g., inflammatory bowel disease) or chronic diarrhea before transplantation were also excluded. The study was performed with the approval of the local ethics committee (protocol number: KEK-ZH-Nr. 2014-0522).

### Data collection

Demographic data including age, gender, cause of end-stage renal disease, and death, if applicable, were collected. With respect to transplantation, the date, initial immunosuppressive regimen, and CMV status were recorded. For characterization of chronic diarrhea, the onset and resolution dates and the accompanying change in body weight were documented. Evidence for an infectious trigger was defined as the

presence of one or more of the following parameters at diarrhea onset: travel history; environmental history for diarrhea; microbiological evidence of a pathogen in a stool sample/culture positivity for *Shigella*, *Salmonella*, or *Campylobacter*; positivity for *Clostridium difficile* toxin; detection of norovirus by polymerase chain reaction (PCR) testing (introduced in 2004); detection of parasites; or detectable CMV in peripheral blood (viral load >200 copies/mL or pp65 antigen positivity at time of diarrhea onset) or in an intestinal biopsy. If available, intestinal histology was included. Type, dose, and change of the immunosuppressive regimen during chronic diarrhea were monitored (particularly regarding anti-proliferative agents such as MMF, EC-MPF, or AZA).

### Statistics

Descriptive statistics of the variables analyzed are presented as mean, ± standard deviation, or ± standard error of the mean. For comparison of independent samples, Student's *t*-test was used. Comparison of related samples was done with the paired *t*-test. Event-free (i.e., diarrhea-free) survival was analyzed using the Kaplan–Meier method. For comparison of seasonal variation, an analysis of variance calculation (Kruskal–Wallis test) was performed. The effect of different treatments on diarrhea resolution, as compared to no intervention, was compared using Fisher's exact test. All analyses were done using Graph Pad Prism Software.

## Results

### Study population

From 2000 to 2010, a total of 726 patients received a kidney transplant (451 from deceased and 275 from living donors) and had at least 1 follow-up visit at the University Hospital of Zurich until July 2014. Median follow-up was 8.25 years (3012 days; interquartile range [IQR] 1787–3858 days). Median age at transplantation was 54 years (IQR 42–59 years). Among all kidney transplant recipients, 65 patients (9%) developed at least 1 episode of chronic diarrhea during the period of observation. Of those, 19 patients were excluded because of having chronic diarrhea already before transplantation ($n = 3$), known inflammatory bowel disease ($n = 4$), MPA-free immunosuppression at diarrhea onset ($n = 2$), incomplete records ($n = 8$), exocrine pancreas insufficiency ($n = 1$), or microscopic colitis ($n = 1$). Thus, 46 patients (6.3% of the cohort) with a total of 51 episodes of chronic diarrhea were included for further analysis (Fig. 1). The immunosuppressive treatment at diarrhea onset included MMF in 45 episodes and EC-MPS in 6 episodes.

Table 1 summarizes the baseline characteristics of these patients. As compared with the whole transplant cohort, no statistical differences were seen in terms of gender ($P = 0.77$), age ($P = 0.79$), or donor type ($P = 0.09$) between the group of transplant recipients with chronic diarrhea versus those without.

### MPA-associated diarrhea is a clinically relevant problem

The clinical course of chronic diarrhea during MPA therapy was typically characterized by acute onset of watery diarrhea, followed by incomplete remission leading to persistent or frequently relapsing bouts of diarrhea. The median duration of chronic diarrhea was 247 days (IQR 64–446 days). The mean overall weight loss from onset to resolution of diarrhea was 5.5 kg (95% confidence interval [CI] −7.6 to −3.5 kg, $P < 0.001$), i.e., 7.3% (95% CI −10.1 to −4.5%) of body weight. Some patients lost >15 kg of their body weight (Figure S1).

### MPA-associated chronic diarrhea is not increased during the first post-transplant year but shows seasonal peaks

To investigate our hypothesis of an infectious trigger of MPA-associated chronic diarrhea, as opposed to a drug

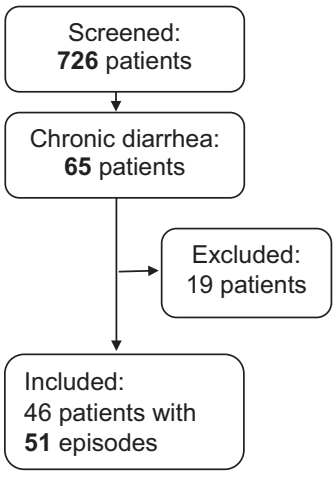



*Fig. 1.* Patient flowchart.

**Baseline characteristics**

| Variable | *N* = 46 |
|---|---|
| Age year: median (interquartile range) | 54 (42–59) |
| Male gender, *n* (%) | 29 (63.0) |
| Cause of end-stage renal disease, *n* (%) | |
| Glomerulonephritis | 11 (23.9) |
| Diabetes mellitus | 1 (2.2) |
| Arterial hypertension | 3 (6.5) |
| Pyelonephritis or interstitial nephritis | 4 (8.7) |
| Polycystic kidney disease | 10 (21.7) |
| Uncertain | 7 (15.2) |
| Other | 10 (21.7) |
| Diabetes mellitus, *n* (%) | 6 (13.0) |
| Donor type, *n* (%) | |
| Living | 11 (23.9) |
| Deceased | 35 (76.1) |
| Transplantation, *n* (%) | |
| First kidney | 37 (80.4) |
| Second/third kidney | 7 (15.2) |
| Kidney + islets | 1 (2.2) |
| ABO incompatible | 1 (2.2) |
| CMV status, *n* (%) | |
| High risk ($D^+/R^-$) | 11 (25) |
| Intermediate risk ($R^+$) | 25 (56.8) |
| Low risk ($D^-/R^-$) | 8 (18.1) |
| Initial post-operative immunosuppression, *n* (%) | |
| CsA + MMF + PDN | 20 (43.5) |
| CsA + MMF + PDN + Basiliximab | 5 (10.9) |
| CsA + MMF + PDN + ATG | 2 (4.3) |
| CsA + EC-MPS + PDN | 2 (4.3) |
| CsA + EC-MPS + PDN + Basiliximab | 1 (2.2) |
| Tac + MMF + PDN | 14 (30.4) |
| Tac + MMF + PDN + Basiliximab | 2 (4.3) |

CMV, cytomegalovirus; D, donor; R, recipient; CsA, cyclosporine; MMF, mycophenolate mofetil; PDN, prednisone; ATG, anti-thymocyte globulin; EC-MPS, enteric-coated mycophenolate sodium; TAC, tacrolimus.

*Table 1*

toxicity effect alone, we first assessed the time-point of diarrhea onset after transplantation. The onset of chronic diarrhea was uniformly distributed throughout the follow-up period (Fig. 2). Of note, the incidence of chronic diarrhea was not particularly high within the first months post transplantation and initiation of MPA

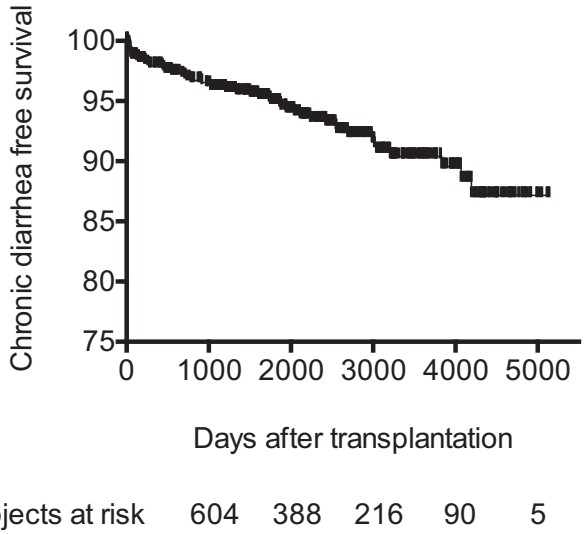



***Fig. 2.*** Incidence of chronic diarrhea after kidney transplantation. Kaplan–Meier estimation of "chronic diarrhea-free" survival for kidney transplant patients during immunosuppressive treatment with mycophenolic acid (MPA).

treatment. Cumulative incidence of chronic diarrhea was 2.0%, 5.1%, and 9.6% at 1, 5, and 10 years, respectively. Furthermore, we observed 2 seasonal peaks, with higher incidence of diarrhea onset in April and in October–November, in contrast to a very low incidence in summer (Fig. 3A and B) ($P = 0.038$). Thus, we did not observe an early peak of chronic diarrhea onset after transplantation, but a seasonal variation.

### Intestinal infections are frequently present at the onset of MPA-associated diarrhea

In a second analysis, we systematically assessed the clinical reports focusing on direct and indirect evidence for infectious triggers at diarrhea onset (Fig. 4). Direct evidence of infectious diarrhea was defined by positive microbiological analysis. We found evidence for bacterial enteritis in 4 episodes (2 *Campylobacter jejuni*, 1 *Salmonella* enteritis, 1 *Mycobacterium tuberculosis*). In addition, *C. difficile* toxin was detected in 2 episodes and parasites in 7 episodes (*Hymenolepis*, *Microsporidia*, *Isospora belli*, and/or *Dientamoeba*). In 15 episodes, norovirus was found in stool specimens; in 7 episodes, CMV was detected in blood at diarrhea onset; and in 6 episodes, CMV colitis was found by immunohistochemistry of intestinal biopsies. Indirect evidence for an infectious diarrhea was defined by highly

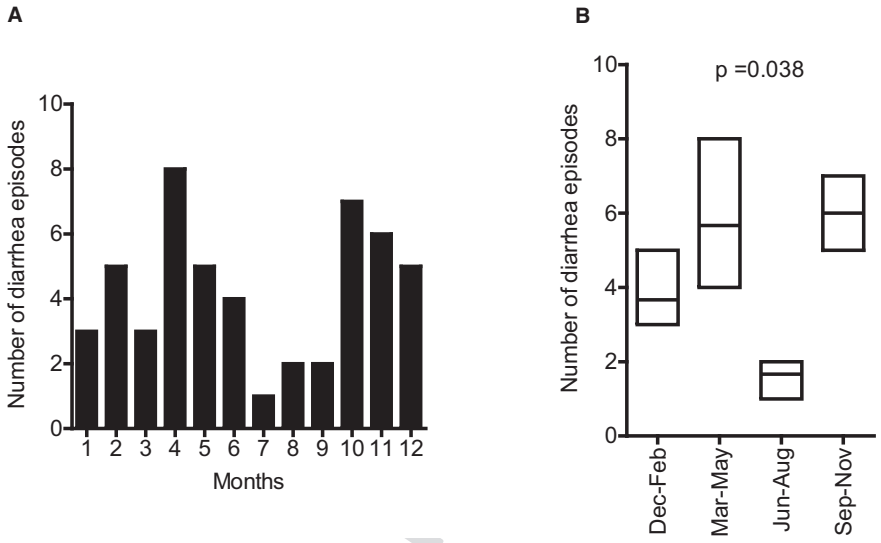



*Fig. 3.* Seasonal distribution of onset of chronic diarrhea episodes. (A) Diarrhea onset depicted for each month. (B) Seasonal clustering of diarrhea onset. Number of diarrhea episodes per season as compared with Kruskal–Wallis test. Boxes depicting maximum, minimum, and mean number of episodes during the observed 3-month seasonal period.

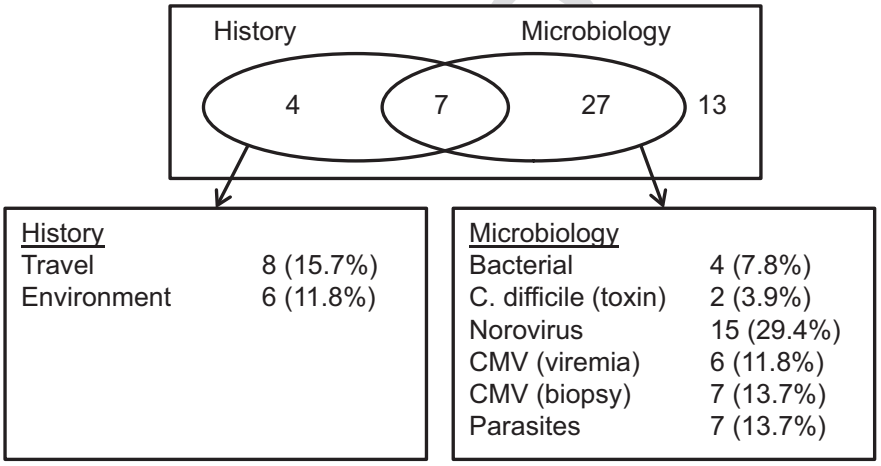



*Fig. 4.* Evidence for infectious triggers at diarrhea onset. All patients with chronic diarrhea are depicted in the rectangular box. The left circle includes patients with indirect evidence for an infection at diarrhea onset (travel history, environmental history). The right circle includes patients with direct microbiological evidence for an infection at diarrhea onset. The overlap shows the patients with both direct and indirect evidence. Multiple infectious triggers were detected for several episodes of chronic diarrhea, as indicated. C., *Clostridium*; CMV, cytomegalovirus.

suggestive reports in the medical history and was present in 11 episodes. In 8 episodes of diarrhea, a recent relevant travel history was reported, and in 6 episodes, similar symptoms were reported in other family members (with some overlap between the 2 criteria). Collectively, and in consideration of overlapping clinical and microbiological results (i.e., some patients had direct and indirect evidence for infectious diarrhea), evidence for an infectious trigger at diarrhea onset was identified in 38 episodes of chronic diarrhea (74.5%). In 34 episodes, this was supported by microbiological analyses, and in 4 episodes, the evidence for infection was only indirect.

Colonoscopy was performed in 31 episodes (60.8%). Intestinal biopsies revealed typical histopathological features of MPA-associated diarrhea in 13 (42%) episodes with increased number of apoptotic cells and typical intestinal crypt distortion; 10 episodes (32.3%) had evidence of predominant inflammation; and in 8 (25.8%), no histopathological changes were described.

### MPA sustains rather than induces chronic diarrhea

Several changes in therapy were attempted to control chronic diarrhea. In general, if possible, antimicrobial

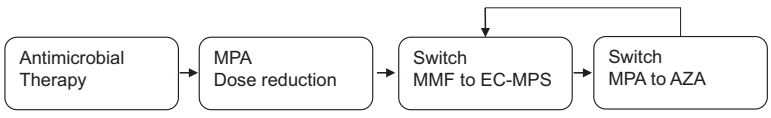
therapy was given first. In case of persistence, changes in immunosuppression such as dose reduction and switch from MMF to EC-MPS or to an MPA-free regimen were attempted to control chronic diarrhea by the treating physicians (Fig. 5A). As a result, chronic diarrhea fully resolved in 45 of 51 episodes (88.2%); in 6 cases (11.8%), diarrhea persisted. In 2 episodes (6.7%), the chronic diarrhea resolved without any intervention. In patients treated with antimicrobial therapy alone, resolution of diarrhea was observed in 19% of episodes. MPA dose reduction was associated with resolution of diarrhea in 22.7% of episodes. Switch from MMF to EC-MPS was followed by resolution of diarrhea in 76.5% of cases ($P < 0.001$) and switch to an MPA-free regimen was followed by resolution of diarrhea in 100% of episodes ($P < 0.001$). Of note, switch to an MPA-free regimen with AZA was significantly more efficient than continuation of MPA ($P = 0.032$) (Fig. 5B and C), and was associated with significantly faster resolution of diarrhea: median time to resolution after switch to AZA: 19 days (IQR 6–35 days) versus 63 days (IQR 11–69 days) after switch from MMF to EC-MPS, $P = 0.0009$ (Figure S2). Interestingly, in 5 cases, MPA (MMF in 1 episode and EC-MPS in 4 episodes) was re-introduced following diarrhea resolution. None of these patients presented a diarrhea relapse after re-exposure to MPA (median follow-up 117 days, IQR 21–743 days), supporting the hypothesis that MPA toxicity alone is not sufficient to explain chronic diarrhea in transplant patients.

## Discussion

Chronic diarrhea is a major problem after transplantation. It substantially reduces quality of life, leads to malabsorption with significant weight loss and oxalate nephropathy (9, 10), and is associated with reduced graft and patient survival (2–5). Although the majority of published studies on post-transplant diarrhea consider acute and chronic diarrhea in general, in this study we focused on chronic diarrhea, defined as diarrhea lasting for >1 month (22). We found a cumulative incidence of chronic diarrhea of 2.0%, 5.1%, and 9.6% at 1, 5, and 10 years after kidney transplantation. This lower incidence, compared with previous reports (5), is likely related to our strict definition of chronic diarrhea aiming at investigating patients with a clinically relevant condition. Therapy for chronic diarrhea in kidney transplant recipients is



A




B

| Treatment category | Resolution per category in % (cases resolved / cases treated) |
|---|---|
| No Change | 6.7 (2/30) |
| Antimicrobial Therapy | 19 (4/21) |
| Dose reduction of IS | 22.7 (5/22) |
| Switch of IS from MMF to EC-MPS | 76.5 (13/17) |
| Switch of IS from MPA to AZA | 100 (21/21) |

C

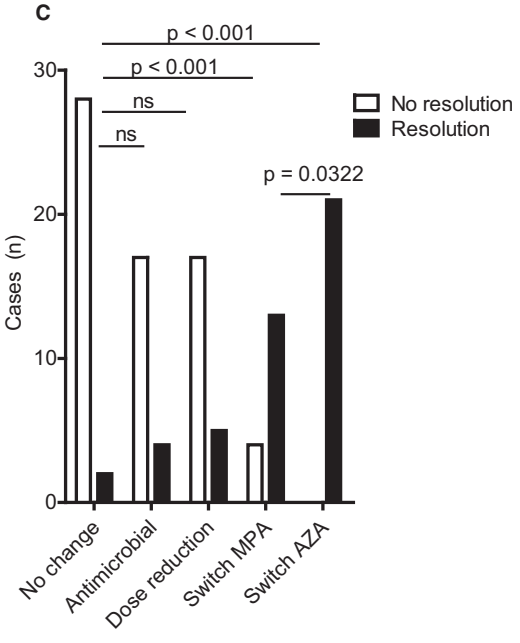



*Fig. 5.* Treatment strategies and related outcomes in episodes of chronic diarrhea during mycophenolic acid (MPA) treatment. (A) General stepwise approach for treating episodes of chronic diarrhea during MPA treatment. (B) Changes in treatment regimen and diarrhea resolution rate per category. (C) Comparison of therapeutic efficacy of the different treatment categories by Fisher's exact test. MMF, mycophenolate mofetil; EC-MPS, enteric-coated mycophenolate sodium; AZA, azathioprine; IS, immunosuppression.

challenging, as etiology often remains unclear (5). In the absence of a specific cause, diarrhea has usually been attributed to MPA toxicity, even though the exact mechanism is unknown (17). This retrospective, observational study in 726 patients with a total of 51 episodes of chronic diarrhea identifies a high incidence of intestinal infections at onset of chronic post-transplant diarrhea and suggests that infections might play an important role in the pathophysiology of chronic post-transplant diarrhea, which is typically attributed to MPA toxicity.

The following findings support the hypothesis of a critical role of an infectious trigger in chronic post-transplant diarrhea:

1 The uniform annual incidence of chronic diarrhea onset after transplantation, with some patients developing diarrhea several years after first exposure to MPA. This suggests that an additional trigger is required for initiating diarrhea. If drug toxicity would be sufficient for induction of chronic diarrhea, a peak incidence in the first months after drug initiation would be expected.
2 The seasonal distribution of diarrhea onset with highest incidence in April and October/November and lowest in July. Coste et al. (8) report a similar seasonal clustering with highest rates of enteric pathogens in stool samples of kidney transplant patients in March and October.
3 Detection of an intestinal infection at the onset of 75% of all episodes of chronic diarrhea, despite the retrospective study design and limited sensitivity of conventional methods to find enteric pathogens. Indeed, a recent study showed that conventional methods detected enteric pathogens in 23% of stool samples only, whereas novel multiplex PCR assays were able to identify enteric pathogens in 72% of stool samples (8). We observed a high incidence of viral pathogens, predominantly of norovirus and CMV infections. This finding is in line with a recent report showing that norovirus and sapovirus infections account for half of the cases of previously classified "diarrhea with unknown causes" and for 30% of the whole cohort of patients suffering from chronic diarrhea (10). Notably, some of the bacteria and parasites identified in the stool at diarrhea onset are not necessarily pathogenic, or classical causes of chronic diarrhea. We speculate that, in the multifactorial complex pathogenesis of chronic diarrhea after transplantation, these microorganisms might indeed damage the intestinal mucosa and trigger intestinal inflammation, but this hypothesis needs to be validated in other studies.
4 The observation that switching back from AZA to MPA was not associated with re-occurrence of diarrhea suggests that drug toxicity is not sufficient for chronic post-transplant diarrhea.

These findings are compatible with a 2-hit mechanism on the intestinal mucosa leading to chronic post-transplant diarrhea, i.e., infection-induced damage combined with reduced repair capacity. MPA impairs pathogen clearance and mucosal repair through its immunosuppressive effects on lymphocytes, the associated hypogammaglobulinemia (23), and inhibition of purine synthesis. Hence, it substantially inhibits epithelial regeneration, especially in the intestine, owing to high local activity because of enterohepatic recirculation. Histological evidence of apoptosis and crypt distortion, known features of MPA-associated colitis (24), was found in 42% of our cases. The crucial role of the integrity of the epithelial lining for resolution of diarrheal symptoms is supported by the description of recovery from diarrhea with reduction of immunosuppression, despite persistence of norovirus shedding, and its reoccurrence after augmentation of the dose (7, 10). This effect of the epithelial lining explains why specific anti-infectious treatment alone may not be sufficient to stop diarrhea, if regeneration of enterocytes is strongly impaired. However, re-introduction of MPA after regeneration of the epithelial lining is safe, if an additional infectious trigger has been eliminated, either by antimicrobial treatment or by restoration of the immunological defense in case of viral infections. In treatment-refractory cases, a transient conversion to an MPA-free regimen with AZA may allow for epithelial recovery and hence resolve chronic refractory post-transplant diarrhea, as evidenced by a 100% resolution rate observed in our cohort. Similar resolution rates have previously been reported after a switch to AZA (10).

The consistent medical records documentation and the long follow-up time strengthen our finding of an additional "infectious" trigger as key element for induction of chronic MPA-associated diarrhea. However, this study has several limitations. Conclusions regarding resolution of MPA-associated diarrhea following changes in immunosuppressive drug regimen are limited by the retrospective design of our study and heterogeneity of treatment changes in patients suffering from chronic diarrhea. As such, Figure 5A shows only the overall stepwise approach of treatment adjustments. Furthermore, we did not routinely monitor MPA drug levels as they are known to show large intra- and inter-individual pharmacokinetic variability (15). Therefore, no correlation could be made between MMF dose

and risk of chronic diarrhea. Similarly, effects of additional immunosuppressive drugs, such as calcineurin inhibitor levels that might increase with diarrhea-associated dehydration and therefore limit clearance of infections, have not been investigated. Hence, owing to lack of drug level monitoring and lack of biopsy results in half of the patients, we cannot exclude that chronic diarrhea in some of our patients was just a result of over-immunosuppression and related reduction of immune defense and not associated with pathognomonic MMF-associated injury of epithelial lining. As such, immunosuppressed patients are generally more likely to suffer from chronic infections (also chronic intestinal infections, i.e., norovirus-related diarrhea). Finally, clearance of infection was not systematically monitored during follow-up.

To conclude, despite the limitations of a retrospective observational study, our data shed light on the causative factors of chronic post-transplant diarrhea and support a new hypothesis for the pathophysiology of this clinically relevant condition. We provide evidence for an infectious agent at diarrhea onset playing an important role, in combination with MPA-based immunosuppression and associated impaired epithelial regeneration, in the development of chronic post-transplant diarrhea. Hence, therapeutically, this implies that both clearance of infection and change or dose reduction in immunosuppression are necessary to resolve chronic post-transplant diarrhea. If confirmed in prospective studies, this hypothesis needs to be validated in interventional studies.

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## Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1**. Weight course during episodes of chronic diarrhea. Comparison of weight at onset of diarrhea and at resolution of chronic diarrhea by paired Student's *t*-test.

**Figure S2.** Time to diarrhea resolution. Time to diarrhea resolution after switching immunosuppression to azathioprine (AZA) as compared to switch from MMF to EC-MPS. Comparison using log-rank test. MMF, mycophenolate mofetil; EC-MPS, enteric-coated mycophenolate sodium.